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Gas-phase fragmentation reactions of protonated benzofuran- and dihydrobenzofuran-type neolignans investigated by accurate-mass electrospray ionization tandem mass spectrometry

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Abstract

We have investigated gas-phase fragmentation reactions of protonated benzofuran neolignans (BNs) and dihydrobenzofuran neolignans (DBNs) by accurate-mass electrospray ionization tandem and multiple-stage (MS^n) mass spectrometry combined with thermochemical data estimated by Computational Chemistry. Most of the protonated compounds fragment into product ions **B** ($[M+H-MeOH]^+$), **C** ($[B-MeOH]^+$), **D** ($[C-CO]^+$), and **E** ($[D-CO]^+$) upon collision-induced dissociation (CID). However, we identified a series of diagnostic ions and associated them with specific structural features. In the case of compounds displaying an acetoxy group at C-4, product ion **C** produces diagnostic ions **K** ($[C-C_2H_2O]^+$), **L** ($[K-CO]^+$), and **P** ($[L-CO]^+$). Formation of product ions **H** ($[D-H_2O]^+$) and **M** ($[H-CO]^+$) is associated with the hydroxyl group at C-3 and C-3', whereas product ions **N** ($[D-MeOH]^+$) and **O** ($[N-MeOH]^+$) indicate a methoxyl group at the same positions. Finally, product ions **F** ($[A-C_2H_2O]^+$), **Q** ($[A-C_3H_6O_2]^+$), **I** ($[A-C_6H_6O]^+$), and **J** ($[I-MeOH]^+$) for DBNs and product ion **G** ($[B-C_2H_2O]^+$) for BNs diagnose a saturated bond between C-7'-C-8'. We used these structure-fragmentation relationships in combination with deuterium exchange experiments, MS^n data, and Computational Chemistry to elucidate the gas-phase fragmentation pathways of these compounds. These results could help to elucidate DBN and BN metabolites in *in vivo* and *in vitro* studies on the basis of ESI-CID-MS/MS data only.

Keywords: benzofuran, computational chemistry, dihydrobenzofuran, fragmentation mechanisms, neolignans

1 INTRODUCTION

Neolignans (NLs) are an important class of natural products derived from the shikimate pathway. They contain two phenylpropanoid (C_6C_3) units linked by two carbon atoms other than β,β' (or 8,8').¹ Dihydrobenzofuran-type neolignans (DBNs) and their correlated benzofuran-type derivatives (BNs) are a group of NLs that display two phenylpropanoid units between positions 7-O-4' and 8-5'.² Interest in DBN and BN skeletons stems from their wide spectrum of biological activities, such as trypanocidal,^{3,4} leishmanicidal,⁵ schistosomicidal,⁶ insecticidal,^{7,8} antioxidant,^{9,10} and anti-inflammatory actions.¹⁰ The biological effects of synthetic and semi-synthetic DBN derivatives have also been extensively investigated.¹⁰

Over the last two decades, electrospray ionization mass spectrometry (ESI-MS) has proven to be a powerful technique to analyze natural products and synthetic low molecular weight compounds. ESI-MS is a soft ionization technique that enables analysis of thermolabile and non-volatile compounds with discrete or no fragmentation.¹¹ ESI-MS combined with tandem (MS/MS) and multiple-stage (MS^n) mass spectrometry can also provide structural information. Combination of ESI-MS/MS and ESI- MS^n data with liquid chromatography (LC) results has been employed to de-replicate and to identify bioactive compounds and metabolism products in complex mixtures.^{11,12} However, elucidating fragmentation pathways and conducting systematic characterization of product ion structures of many classes of synthetic and natural products still represent a major challenge regarding metabolism product identification during *in vivo* and *in vitro* studies.¹³

Despite the broad scope of DBN and BN biological activities, studies dedicated to the fragmentation of these compounds by ESI-MS/MS are still scarce in the literature. Len and co-workers employed ESI-MS/MS to identify diastereoisomeric dihydrobenzofuran-1-yl thiamines and a novel 1,3-dihydrobenzofuran nucleoside on the basis of product ion spectra of singly protonated and sodium complex of selected precursor ions by low-energy collision-induced dissociation (CID).¹⁴ Yang and co-workers studied fragmentation of coumestans, pterocarpenes, benzofurans, and isoflavones present in *Hedysarum multijugum* and identified twenty-nine compounds in the

corresponding methanol extract, including five benzofurans, which exhibited characteristic losses, like CO and H₂O.¹⁵ Kang and co-workers described that H₂O loss from protonated benzofuran glycosides present in *Saposhnikovia divaricata* root extracts involves dihydrofuran ring cleavage.¹⁶ Morreel and co-workers reported that water (18 Da) loss followed by C₈H₈ elimination in competition with C₈H₈ or even CH₂O losses from the precursor ion is the main fragmentation pathway for deprotonated dihydrodehydrodiconiferyl alcohol.¹⁷ Similarly, Boldizsár and co-workers reported that water elimination from the *Cirsium vulgare* fruit benzofuran neolignan balanophonin also involves benzofuran ring opening, with aromatic chain loss followed by aliphatic chain elimination.¹⁸

As part of our ongoing project on the fragmentation reactions of natural products¹⁹⁻²³ and synthetic compounds,^{24,25} we investigate the fragmentation pathways of a series of synthetic DBNs and BNs by electrospray ionization accurate-mass tandem mass spectrometry and MSⁿ combined with thermochemical data estimated by Computational Chemistry.

2 MATERIALS AND METHODS

2.1 Synthesis

The DBNs investigated in this study were synthesized as reported previously.^{6,26,27} Briefly, the core structure of compounds **1–8** was obtained by oxidative coupling of coumaric (**a**), ferulic (**b**), and caffeic (**c**) methyl esters promoted by Ag₂O in acetone/benzene (5:8) mixture. The reaction was conducted inside a two-neck flask covered with aluminum foil and equipped with a magnetic stirrer and a nitrogen gas tube at room temperature, for 20 h (Scheme 1). Products were purified by column chromatography (2.2 x 100 cm, silica gel 60, 0.040-0.063 mm) with hexane/ethyl acetate (2:1) as eluent, which gave compounds **1**, **2**, and **3** in 34%, 40%, and 12% yield, respectively. Acetylated products **4** and **5** were obtained by reacting compounds **1** and **2** with Ac₂O/pyridine in a two-neck flask equipped with a magnetic stirrer and under N₂ atmosphere at room temperature, for 48 h. Next, the solution was evaporated with toluene at reduced pressure to provide an azeotropic solution. This procedure afforded compounds **4** and **5** in 96% and 82% yield, respectively. Compound **1** was

oxidized in a three-neck flask equipped with a magnetic stirrer, under reflux and N₂ atmosphere, for 22 h. DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone) and dry dioxane were added as oxidant agent and solvent, respectively. The solution was cooled, and DDQ was filtered off and evaporated under reduced pressure. The resulting product was purified by column chromatography (1.2 x 60 cm, silica gel 60, 0.040-0.063 mm) with hexane/ethyl acetate (1:2) as eluent, which yielded compound **6** (74%). Compounds **1** and **6** were reduced by dissolution in dry acetone and addition to Pd/C (5%). The flask was sealed and stirred under 60-psi H₂ pressure at room temperature, for 2 h. The mixture was filtered to remove the catalyst. After that, the mixture was evaporated under reduced pressure, which afforded compounds **7** (96%) and **8** (94%). All structures were confirmed by NMR analyses (see support information).

2.2 Mass spectrometry analysis

Compounds **1–8** were analyzed by accurate mass electrospray ionization tandem mass spectrometry on an microTOF-Q (Bruker Daltonics, USA) instrument fitted with an electrospray ionization (ESI) source operating in the positive ion mode. Samples were dissolved in MeOH/H₂O (70:30 v/v). Then, traces of 0.1% formic acid were added, and the samples were directly infused into the ionization source at a flow rate of 3 µL/min. Deuterium exchange experiments were carried out by dissolving the compounds in MeOH/D₂O (70:30 v/v), which was followed by further infusion into the ionization source under the same conditions. Sodiated trifluoroacetic acid was used as internal standard for calibration. Source block and de-solvation temperatures were set to 150 °C. Voltage values applied at the capillary and cone were optimized at 3.0 kV and 500 V, respectively, which maximized the relative intensity of the peaks corresponding to the protonated molecule. The protonated molecule [M + H]⁺ of each compound was selected as precursor ion and activated by collision-induced dissociation (CID) with N₂ as collision gas at energy values ranging between 5 and 40 eV.

MSⁿ analyses of the studied compounds were performed with the same dilution that was used during the accurate mass analyses. The compounds were directly infused into the Ion trap Bruker Amazon SL equipment at a flow rate of 5 µL/min. N₂ was applied as nebulizing (7 psi) and drying gas

(8 mL/min, 200 °C). The capillary high voltage was 3.5 kV, and the end plate offset was set to 500 V for masses ranging from 50 to 900 m/z .

2.3 Computational methods

All molecules were optimized with the B3LYP/6-31G(d) model²⁸ by using Gaussian 03 suite programs.²⁹ The B3LYP method was chosen to calculate the selected parameters, which provided accurate measurements at minimum computational cost.³⁰ Vibrational frequencies indicated that all structures were minima in the potential energy surface. All the structures were visualized with the J.Mol 9.0 software.³¹ Protonation sites were suggested on the basis of PA.³² PA was obtained by variations in enthalpies. For a protonation reaction $M + H^+ \rightarrow MH^+$, enthalpy was considered to be 1.48 kcal/mol.^{33,34} Hence, it was possible to propose the most stable ions and the fragmentation pathways suggested by enthalpies and Gibbs energies at 298.15 K. No transition states were calculated for the fragmentation mechanisms; only the relative enthalpies/Gibbs energies were obtained because CID was assumed to supply the minimum energy for fragmentation. In addition, the Gibbs energies were used with caution because equilibrium was not reached during the CID experiments.^{34,35}

3 RESULTS AND DISCUSSION

3.1 Structure-fragmentation correlation

Figure 1 shows the product ion spectra of protonated compounds **1–8** at collision energy (E_{lab}) of 10 eV. This energy maximizes the number of peaks in the product spectrum with relative intensity higher than 5% and reduces the precursor ion relative intensity to below 50% without promoting extensive fragmentation (see Supporting Information). Table 1 lists the assignments of the main product ions (relative intensity higher than 5%) at this collision energy. Product ions **B** and **C** are common to all DBNs **1–8** and result from one and two losses of two MeOH (32 Da) from the precursor ion, respectively (Scheme 2). These product ions are the most intense in the product ion spectra of compounds **1, 2, 4, 6, and 8** obtained at 10 eV. In addition, one and two CO (28 Da) losses from

product ion **C** generate product ions **D** and **E**, respectively, which are also common to compounds **1–3** and **6–8**.

We identified a series of product ions that can diagnose specific structural features of the analyzed compounds, as depicted in Scheme 2. In the case of compounds **4** and **5**, which bear an acetoxy group at C4, ketene elimination (42 Da) from product ion **C** can precede CO elimination, to give product ion **K** and its derivatives **L** ($[\mathbf{K}-\text{CO}]^+$) and **P** ($[\mathbf{L}-\text{CO}]^+$). For compound **3**, the hydroxyl group at C-3 and C-3' is associated with water elimination from product ion **D** and further CO loss, to afford product ions **H** ($[\mathbf{D}-\text{H}_2\text{O}]^+$) and **M** ($[\mathbf{H}-\text{CO}]^+$), respectively. On the other hand, the methoxyl groups at C-3 and C-3' in compound **2** participate in the formation of product ions **N** (m/z 291) and **O** (m/z 259), which originate from one and two MeOH eliminations from product ion **D**.

Regarding compound **8**, ketene (42 Da) elimination from product ion **B** and consequent product ion **G** (m/z 281) formation is due to the presence of a saturated bond between C-7' and C-8' and unsaturation between C-7 and C-8. Product ion **Q** (m/z 283) results from direct methyl acetate ($\text{C}_3\text{H}_6\text{O}_2$) elimination from protonated compound **7**, indicating that a double bond does not exist between C-7 and C-8 and between C-7' and C-8'. Furthermore, product ions **F** ($[\mathbf{A}-\text{H}_2\text{O}]^+$), **I** ($[\mathbf{A}-\text{C}_6\text{H}_6\text{O}]^+$), and **J** ($[\mathbf{I}-\text{CH}_3\text{OH}]^+$), which only appear in the product ion spectrum of compound **7**, indicate the presence of a saturated bond at the same position. MSⁿ experiments fully support the fragmentation pathways depicted in Scheme 2 (see Supporting Information).

3.2 Protonation site search

Given that DBNs can be protonated at many different sites, we analyzed the literature to determine the more susceptible protonation structures. In his remarkable paper, Bouchoux described some relationships concerning the basicity of different polyfunctionalized molecules.³⁶ Hunter and Lias established that conjugated carbonyl groups and/or presence of electron-releasing groups in α,β -unsaturated carbonyl moieties tend to increase PA.³⁷ More recently, Dias and co-workers demonstrated that the carbonyl oxygen of α,β -unsaturated carbonyl groups is the most susceptible protonation site in a series of 2-arylbenzofurans.²⁵ On the basis of these studies, the oxygen atoms of

the structure of compounds **1–8** are possible protonation sites. Therefore, we estimated their PA values by computational methods, at the B3LYP/6-31G(d) level of theory. We also tested the B3LYP/6-31+G(d,p) level of theory, but we did not observe remarkable differences between these two base systems, so we opted for the one with the smallest computational cost.

The data estimated by computational quantum chemistry indicated that PA is higher on carbonyl oxygen atoms or furan ring oxygen atoms, as illustrated in Figure 2. Moreover, comparison between the PA values of compounds **1–8** showed that the double bonds conjugated to carbonyl groups boost the carbonyl oxygen atom PA, as previously described in the literature.³⁷ Thus, except for compound **8**, for which O-9 is estimated to be the site that is the most susceptible to protonation, O-9' is the site that is the most prone to protonation in all the investigated compounds (Figure 2).

3.3 General fragment ion formation

Product ions **B** and **C** are common to all the DBNs evaluated in this study. In principle, product ion **B** formation from the precursor ion can occur through two possible mechanisms: (**I**) MeOH elimination preceded by proton migration from O-9' (the most susceptible site to protonation, as evidenced by the PA values) to O-10', followed by charge-induced C9'-O10' cleavage and acylium ion formation;²⁷ or (**II**) charge-remote MeOH elimination without proton migration.¹³ However, data from deuterium exchange experiments (see supporting information) demonstrated that product ion **B** stems from MeOD elimination (33 Da) from the precursor ion, which strongly supports mechanism **I**, as shown in Scheme 3.^{38,39} Theoretical data estimated at the B3LYP/6-31G(d) level of theory revealed that the enthalpy involved in proton migration (mechanism **I**) is 18.5-10.0 kcal/mol. The CID process ($E_{lab} = 10$ eV) can supply this energy content.³⁵ In contrast, deuterium exchange experiments showed that product ion **C** results from MeOH (and not MeOD) elimination from product ion **B** to give product ion **C**, except for compound **6**. This evidences that a deuterium/proton participates in product ion **C** formation. Hence, for compounds **1–5**, product ion **C** formation involves a charge-remote hydrogen rearrangement with H-8 participation, as shown in Scheme 3.⁴⁰ The peak ascribed to product ion **C** is

the most intense in the product ion spectra of compounds **1**, **2**, and **4** ($\Delta H = 30.3$, 30.7 , and 31.0 kcal/mol, respectively).

Product ion **D** ($[\text{C-CO}]^+$) formation is common to all the analyzed DBNs, except for compounds **4** and **5**. For compounds **1–3**, product ion **D** formation is preceded by product ion **C** conversion to the intermediate **C1**, from which a CO molecule is eliminated (Scheme 3). Similar reactions to **C1** decarbonylation to produce product ion **D** ($\Delta H_{\text{C} \rightarrow \text{D}} = 13.4\text{--}22.2$ kcal/mol) as well as product ion **E** formation from product ion **D** ($\Delta H_{\text{D} \rightarrow \text{E}} = 34.2\text{--}47.5$ kcal/mol) have been extensively described for natural products.^{41–47} In the product ion spectrum of compound **3**, the peak attributed to product ion **D** is the most intense. This fact could be associated with the presence of hydroxyl groups at C-4, C-3, and C-3', which could also participate in product ion **C** tautomerization, thereby increasing the product ion **D** relative intensity. Additionally, product ion **B** formation from the precursor ion is energetically favorable in compound **3** as compared to compounds **1–5** ($\Delta H = 12.0$ kcal/mol; $\Delta G = 1.2$ kcal/mol), whereas fragmentation of product ions **B** and **C** to give product ions **C** and **D**, respectively, is similar for all the compounds. Therefore, the increased number of product ion **C** that can be converted to product ion **D** could account for the high relative intensity of product ion **D** at $E_{\text{lab}} = 10$ eV for compound **3** (Scheme 3). Regarding product ion **E**, passing through intermediate **D1** requires high energy content. Besides that, in the case of compounds **2** and **3**, the presence of competitive pathways explains the low product ion **E** relative intensities in the product ion spectra of these compounds.

For compound **6**, product ion **C** formation follows a different pathway as compared to the other compounds, in which the hydrogen of the phenol hydroxyl at C-4 is involved (Scheme 4). However, product ion **B** from compound **6** affords the most intense peak in the product ion spectrum for collision energies between 5 and 25 eV (Figure 1). This ion is relatively more stable than the corresponding product ion **B** of the other compounds of the series due to extended conjugation, as evidenced by the lower ΔH and ΔG values for its formation from product ion **A** (23.6 and 11.1 kcal/mol, respectively). Consequently, in the case of compound **6**, product ion **C** formation from product ion **B** requires higher ΔH and ΔG values (52.4 kcal/mol and 39.7 kcal/mol, respectively) as compared to the other compounds ($\Delta H = 30.3\text{--}34.6$ kcal/mol and $\Delta G = 17.8\text{--}21.2$ kcal/mol). In

contrast to compounds **1**, **2**, and **3**, in compound **6** product ion **D** formation occurs by means of a charge-remote decarbonylation from the product ion **C** quinonemethide moiety without participation of intermediates (e.g., **C1**), as shown in Scheme 4. This difference between mechanisms is consistent with ΔH values to form product ion **D** from product ion **C** for compounds **6** ($\Delta H_{C \rightarrow D} = 17.4$ kcal/mol), **1** ($\Delta H_{C \rightarrow D} = 30.0$ kcal/mol), **2** ($\Delta H_{C \rightarrow D} = 20.1$ kcal/mol), and **3** ($\Delta H_{C \rightarrow D} = 27.0$ kcal/mol). On the other hand, product ion **E** formation for compound **6** (Scheme 4) occurs with charge migration and vinylic cation formation²⁵ ($\Delta H_{D \rightarrow E} = 74.4$ kcal/mol), whereas product ion **E** formation for compounds **1–5** involves a charge remote process (Scheme 4).

Lack of a double bond between C-7'-C-8' in compound **7** precludes intermediate **C1** formation. In this case, hydride migration assists CO (28 Da) elimination from product ion **C**, to produce benzyl-type cation **D2** ($\Delta H = -15.8$ kcal/mol, $\Delta G = -26.1$ kcal/mol), as shown in Scheme 4. The aromatic ring π cloud of electrons can also assist CO elimination from product ion **C**, to produce benzyl-type cation **D2'** ($\Delta H = 11.4$ kcal/mol, and $\Delta G = 2.2$ kcal/mol), or CO elimination even occur by means of a single heterolytic C8'-C9' bond cleavage (Scheme 5). In the latter case, the resulting primary carbocation **C'** is spontaneously converted to intermediate **D2''** during geometry optimization ($\Delta H_{C' \rightarrow D2''} = 0.0$ kcal/mol, $\Delta G_{C' \rightarrow D2''} = -9.4$ kcal/mol). However, intermediate **D2** formation is more energetically favorable than intermediate **D2'** or **D2''** formation. Despite the relatively low ΔH and ΔG values involved in intermediate **D2** formation from product ion **C**, its relative intensity in the spectrum of compound **7** at $E_{lab} = 10$ eV is low because formation of product ion **B** and its derivatives **C** and **D** competes with product ion **I** (m/z 263) formation from the precursor ion, as further discussed in this paper. On the other hand, product ion **E1** formation from intermediate **D2** is endothermic and endergonic ($\Delta H = 31.2$ kcal/mol; $\Delta G = 19.2$ kcal/mol). This is observed for compound **7** only when collision energies are higher than 35 eV.

With respect to compound **8**, PA values revealed that protonation at O-9 (Figure 2) is 5.0 kcal/mol more favorable as compared to protonation at O-9'. Proton migration from O-9 to O-10 ($\Delta H = 20.6$ kcal/mol) precedes product ion **B** formation from product ion **A**, as shown in Scheme 6. High product ion **B** relative intensity of can be interpreted in terms not only of the low ΔH and ΔG

values (17.7 and 3.2 kcal/mol, respectively) involved in its formation, but also of the high ΔH and ΔG values for its decomposition into product ion **C** (44.8 and 33.1 kcal/mol, respectively). Contrary to compounds **1–7**, in compound **8**, product ion **D4** formation from product ion **C** ($\Delta H = 33.0$ kcal/mol and $\Delta G = 21.6$ kcal/mol) and product ion **E** formation from product ion **D5** ($\Delta H = 21.6$ kcal/mol and $\Delta G = 9.5$ kcal/mol) take place by charge-remote decarbonylation (Scheme 6).

3.4 Diagnostic product ion formation

The presence of an acetyl group in structures **4–5** refers to product ion **K** ($C-C_2H_2O$) formation. Ketene eliminations have been reported to result from remote hydrogen rearrangements or McLafferty-type rearrangements.¹³ Product ion **K** formation from product ion **C** is endergonic and endothermic for compounds **4** ($\Delta H = 53.5$ kcal/mol and $\Delta G = 41.8$ kcal/mol) and **5** ($\Delta H = 50.6$ kcal/mol and $\Delta G = 38.6$ kcal/mol) because this fragmentation process involves aromaticity loss (Scheme 7). Product ions **L** (**K**–CO) and **P** (**L**–CO) also diagnose acetyl in compounds **4–5**, but they arise at collision energies higher than 25 eV, which fully agrees with the high ΔH and ΔG values involved in their formation.

Concerning compound **8**, ketene elimination from product ion **B** (m/z 323) results in product ion **G** (m/z 281). Formation is associated with the presence of a single bond between C-7' and C-8'. In this case, ketene elimination results from acylium ion **B1** C-7'–C-8' bond cleavage (Scheme 7). Product ion **G** formation from intermediate **B1** ($\Delta H = 3.7$ kcal/mol and $\Delta G = -7.8$ kcal/mol) competes with product ion **C** formation from product ion **B** ($\Delta H = 44.8$ kcal/mol and $\Delta G = 33.1$ kcal/mol). However, product ion **B** formation from precursor ion **A** ($\Delta H = 17.7$ kcal/mol and $\Delta G = 3.2$ kcal/mol, Scheme 6) is energetically more favorable than product ion **B1** formation from product ion **A** ($\Delta H = 48.7$ kcal/mol and $\Delta G = 36.0$ kcal/mol, Scheme 7). Consequently, product ion **C** relative intensity is higher as compared to product ion **G** the relative intensity.

Product ions **F**, **I**, **J**, and **Q** only emerged in the product ion spectra of protonated compound **7** and are associated with single bonds between C-7–C-8 and C-7'–C-8'. Formation of product ions **F**, **I**, and **J** compete with product ion **B** formation from product ion **A** (Scheme 2) and requires proton

migration between O-9 and O-4' ($\Delta H = 0.4$ kcal/mol) to produce intermediate ion **A2**. **A2** decomposition into product ion **Q** (m/z 283) involves hydrogen rearrangement and consequent C-7'–C-8' bond cleavage (pathway I, Scheme 8). High product ion **Q** relative intensity can be interpreted in terms of the low ΔH and ΔG values associated with its formation ($\Delta H = 5.9$ kcal/mol and $\Delta G = -6.6$ kcal/mol). On the other hand, pathway II occurs by product ion **A2** conversion to intermediate ion **A3** ($\Delta H = 16.4$ kcal/mol and $\Delta G = 17.8$ kcal/mol) and further five-membered ring opening to produce the intermediate benzylic carbocation **A4** ($\Delta H = -25.4$ kcal/mol and $\Delta G = -25.6$ kcal/mol). Diagnostic ion **I** (m/z 263), which is the base peak in the spectrum of **7**, originates from C₆H₅OH loss from product ion **A4** ($\Delta H = 12.0$ kcal/mol and $\Delta G = 0.0$ kcal/mol) by means of a charge remote hydrogen rearrangement,⁴⁸ as shown in Scheme 7 (path **IIa**). Further MeOH elimination from product ion **I** yields product ion **J** ($\Delta H = 24.3$ kcal/mol and $\Delta G = 12.5$ kcal/mol). Additionally, path **IIb** produces product ion **F** (m/z 339) from product ion **A4** by means of a remote hydrogen rearrangement and H₂O (18 Da) loss ($\Delta H = 13.6$ kcal/mol and $\Delta G = 2.4$ kcal/mol).⁴⁹ The relatively low ΔH and ΔG values involved in the formation of product ions **F**, **I**, **J**, and **Q** makes this pathway energetically more favorable as compared to formation of product ions **B** ([**A**–CH₃OH]⁺), **C** ([**B**–CO]⁺), and **D** ([**C**–CO]⁺).

The methoxyl and hydroxyl substituents at C-3 and C-3' of compounds **2** and **3**, respectively, could be distinguished on the basis of the diagnostic ions formed from product ion **D**. For compound **3**, product ions **H** (m/z 277, [**D**–H₂O]⁺) and **M** (m/z 249, [**H**–CO]⁺) originate from product ion **D** by remote hydrogen rearrangement and further decarbonylation^{46, 50} ($\Delta H = 47.8$ kcal/mol and $\Delta G = 35.7$ kcal/mol), as shown in Scheme 9. Product ion **H** formation from product ion **D** ($\Delta H = 77.6$ kcal/mol and $\Delta G = 62.9$ kcal/mol)⁴⁸ competes with product ion **E** formation ($\Delta H = 47.5$ kcal/mol and $\Delta G = 35.2$ kcal/mol). Nevertheless, although product ion **H** formation is energetically less favorable as compared to product ion **D**, both product ions have low relative intensity in the product ion spectrum of compound **2**. In addition, for DBN **2**, diagnostic ions **N** ([**D**–CH₃OH]⁺) and **O** ([**N**–CH₃OH]⁺) stem from two consecutive remote hydrogen rearrangements, as depicted in Scheme 9. However, product ion **N** formation from product ion **D** ($\Delta H = 125.0$ kcal/mol and $\Delta G = 96.9$ kcal/mol) is also less

favorable than product ion **E** formation from product ion **D** ($\Delta H = 43.2$ kcal/mol and $\Delta G = -11.9$ kcal/mol).

•CH₃ elimination under ESI-CID-MS/MS conditions has been reported for compounds that display methoxy groups at their aromatic rings or polyunsaturated systems.^{51,52} However, compounds **2** and **5** did not undergo •CH₃ eliminations. To understand this apparently unexpected result, we used Computational Quantum Chemistry to estimate the ΔH and ΔG values associated with •CH₃ elimination from the two methoxy groups in protonated compound **2** (see Scheme 10). Comparison between the ΔH (53.6–64.9 kcal/mol) and ΔG (40.6–52.0 kcal/mol) values obtained for •CH₃ elimination to the ΔH and ΔG values obtained for methanol elimination ($\Delta H=28.3$ kcal/mol; $\Delta G=16.9$ kcal/mol) revealed that $[M+H-\bullet CH_3]^+$ formation is energetically less favorable as compared to $[M+H-MeOH]^+$ formation. At least in principle, differences between the ΔH and ΔG values could explain the absence of product ion $[M+H-\bullet CH_3]^+$ in the product ion spectrum of compound **2**.

4 CONCLUSIONS

Formation of product ions **B** ($[M+H-MeOH]^+$), **C** ($[B-MeOH]^+$), **D** ($[C-CO]^+$), and **E** ($[D-CO]^+$) are the major fragmentation routes of protonated dihydrobenzofuran-type and benzofuran-type neolignans displaying methyl ester functionalities at C-9 and C-9'. However, accurate-mass data, MSⁿ and deuterium exchange experiments, and thermochemical data estimated by Computational Chemistry revealed that formation of these major product ions follows different fragmentation pathways, where the presence or absence of double bonds at specific positions and the presence of acetyl, methoxyl, and methyl substituents play a key role. These data could be useful to identify *in vivo* or *in vitro* metabolism products of these dihydrobenzofuran and benzofuran neolignans by ESI-CID-MS/MS, which are actually underway.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Table 1. Data from the product ion spectra of protonated compounds **1–8** (ESI-QTOF, $E_{lab} = 10$ eV).

	<i>m/z</i> Observed	Theoretical <i>m/z</i>	D ₂ O Experiments	Error (ppm)	Formula of the ion	Assignment
1	355.1167 (8)	355.1176	357	-2.5	C ₂₀ H ₁₉ O ₆ ⁺	A ([M+H] ⁺)
	323.0911 (14)	323.0914	324	-0.9	C ₁₉ H ₁₅ O ₅ ⁺	B (A –CH ₃ OH)
	291.0654 (100)	291.0652	292	+0.7	C ₁₈ H ₁₁ O ₄ ⁺	C (B –CH ₃ OH)
	263.0699 (71)	263.0703	264	-1.5	C ₁₇ H ₁₁ O ₃ ⁺	D (C –CO)
	235.0752 (12)	235.0754	—	-0.9	C ₁₆ H ₁₁ O ₂ ⁺	E (D –CO)
2	415.1358 (5)	415.1387	417	-7.0	C ₂₂ H ₂₃ O ₈ ⁺	A ([M+H] ⁺)
	383.1106 (22)	383.1125	384	-5.0	C ₂₁ H ₁₉ O ₇ ⁺	B (A –CH ₃ OH)
	351.0851 (100)	351.0863	352	-3.4	C ₂₀ H ₁₅ O ₆ ⁺	C (B –CH ₃ OH)
	323.0910 (52)	323.0914	—	-1.2	C ₁₉ H ₁₅ O ₅ ⁺	D (C –CO)
	295.0960 (15)	295.0965	—	-1.7	C ₁₈ H ₁₅ O ₄ ⁺	E (D –CO)
	291.0649 (7)	291.0652	—	-1.0	C ₁₈ H ₁₁ O ₄ ⁺	N (D –CH ₃ OH)
	259.0395 (5)	259.0390	—	+1.9	C ₁₇ H ₇ O ₃ ⁺	O (N –CH ₃ OH)
3	387.1049 (8)	387.1074	391	-6.5	C ₂₀ H ₁₉ O ₈ ⁺	A ([M+H] ⁺)
	355.0817 (14)	355.0812	358	+1.4	C ₁₉ H ₁₅ O ₇ ⁺	B (A –CH ₃ OH)
	323.0546 (93)	323.0550	326	-1.2	C ₁₈ H ₁₁ O ₆ ⁺	C (B –CH ₃ OH)
	295.0591 (100)	295.0601	298	-3.4	C ₁₇ H ₁₁ O ₅ ⁺	D (C –CO)
	267.0648 (10)	267.0652	—	-1.5	C ₁₆ H ₁₁ O ₄ ⁺	E (D –CO)
	277.0494 (8)	277.0495	—	-0.4	C ₁₇ H ₉ O ₄ ⁺	H (D –H ₂ O)
	249.0543 (5)	249.0546	—	-1.2	C ₁₆ H ₉ O ₃ ⁺	M (H –CO)
4	397.1269 (5)	397.1282	398	-3.3	C ₂₂ H ₂₁ O ₇ ⁺	A ([M+H] ⁺)
	365.1024 (22)	365.1020	365	+1.1	C ₂₁ H ₁₇ O ₆ ⁺	B (A –CH ₃ OH)
	333.0755 (100)	333.0757	333	-0.6	C ₂₀ H ₁₃ O ₅ ⁺	C (B –CH ₃ OH)
	291.0653 (73)	291.0652	—	+0.3	C ₁₈ H ₁₁ O ₄ ⁺	K (C –C ₂ H ₂ O)
	263.0701 (13)	263.0703	—	-1.5	C ₁₇ H ₁₁ O ₃ ⁺	L (K –CO)
	235.0750 (5)	235.0754	—	-0.9	C ₁₆ H ₁₁ O ₂ ⁺	P (L –CO)
5	457.1476 (2)	457.1493	458	-3.7	C ₂₄ H ₂₅ O ₉ ⁺	A ([M+H] ⁺)
	425.1232 (5)	425.1231	425	+0.2	C ₂₃ H ₂₁ O ₈ ⁺	B (A –CH ₃ OH)
	393.0959 (87)	393.0969	393	-2.5	C ₂₂ H ₁₇ O ₇ ⁺	C (B –CH ₃ OH)
	351.0855 (100)	351.0869	351	-4.0	C ₂₀ H ₁₅ O ₆ ⁺	K (C –C ₂ H ₂ O)
	323.0927 (5)	323.0914	—	+5.9	C ₁₉ H ₁₅ O ₅ ⁺	L (K –CO)
6	353.1001 (5)	353.1020	355	-5.4	C ₂₀ H ₁₇ O ₆ ⁺	A ([M+H] ⁺)
	321.0748 (100)	321.0757	322	-2.8	C ₁₉ H ₁₃ O ₅ ⁺	B (A –CH ₃ OH)
	289.0481 (39)	289.0495	289	-4.8	C ₁₈ H ₉ O ₄ ⁺	C (B –CH ₃ OH)
	261.0529 (32)	261.0546	—	-6.5	C ₁₇ H ₉ O ₃ ⁺	D (C –CO)
	233.0589 (13)	233.0597	—	-3.4	C ₁₆ H ₉ O ₂ ⁺	E (D –CO)
7	357.1330 (20)	357.1333	359	-0.8	C ₂₀ H ₂₁ O ₆ ⁺	A ([M+H] ⁺)
	339.1209 (40)	339.1227	—	-5.3	C ₂₀ H ₁₉ O ₅ ⁺	F (A –H ₂ O)
	325.1056 (38)	325.1071	326	-4.6	C ₁₉ H ₁₇ O ₅ ⁺	B (A –CH ₃ OH)
	293.0810 (44)	293.0808	294	-2.7	C ₁₈ H ₁₃ O ₄ ⁺	C (B –CH ₃ OH)
	283.0968 (55)	283.0965	—	+1.1	C ₁₇ H ₁₅ O ₄ ⁺	Q (A –C ₃ H ₆ O ₂)
	265.0862 (40)	265.0859	—	+1.1	C ₁₇ H ₁₃ O ₃ ⁺	D (C –CO)
	263.0914 (100)	263.0914	264	0.0	C ₁₄ H ₁₅ O ₅ ⁺	I (A –C ₆ H ₆ O)
	231.0653 (40)	231.0652	—	+0.4	C ₁₃ H ₁₁ O ₄ ⁺	J (I –CH ₃ OH)
8	355.1159 (15)	355.1176	357	-4.8	C ₂₀ H ₁₉ O ₆ ⁺	A ([M+H] ⁺)
	323.0904 (100)	323.0914	324	-3.1	C ₁₉ H ₁₅ O ₅ ⁺	B (A –CH ₃ OH)
	291.0640 (19)	291.0652	—	-4.1	C ₁₈ H ₁₁ O ₄ ⁺	C (B –CH ₃ OH)
	281.0819 (25)	281.0808	—	+3.9	C ₁₇ H ₁₃ O ₄ ⁺	G (B –C ₂ H ₂ O)
	263.0719 (21)	263.0703	—	+6.1	C ₁₇ H ₁₁ O ₃ ⁺	D (D –CO)
	235.0768 (12)	235.0754	—	+6.0	C ₁₆ H ₁₁ O ₂ ⁺	E (F –CO)

Figure captions

SCHEME 1. Synthesis of dihydrobenzofuran-type neolignan (DBN) and benzofuran-type (BN) derivatives. (I) Ag_2O , $(\text{CH}_3)_2\text{CO}/\text{C}_6\text{H}_6$, reaction time = 20 h; 34%, 40%, and 12% yield for compounds **1**, **2**, and **3**, respectively; (II) Ac_2O , Pyr, reaction time = 48 h; 96% and 82% yields for compounds **4** and **5**, respectively; (III) DDQ, dioxane, 105 °C, reaction time = 22 h; 74% yield for compound **6**; (IV) H_2 , Pd/C, 60 psi, reaction time = 2 h; 96% and 94% yields for compounds **7** and **8**, respectively.

SCHEME 2. Structure-fragmentation relationships for protonated DBNs **1–5** and **7** and BNs **6** and **8**.
*Observed only in collision energies higher than 10 eV.

FIGURE 1. Product ion spectrum of protonated DBN derivatives (Q-TOF, N_2 , $E_{\text{lab}} = 10$ eV)

FIGURE 2. Proton affinity (PA, kcal/mol) values for compounds **1–8**, as calculated at the B3LYP/6-31G(d) level.

SCHEME 3. Formation of product ions **B** and **C** for compounds **1–5** and of product ions **D** and **E** for compounds **1–3**. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 4. Formation of product ions **B**, **C**, **D**, and **E** from protonated compounds **6** and **7**. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 5. Formation of product ions **D2'** and **D2''** from **C** for compound **7**. **Enthalpies** and *Gibbs energies* are in kcal/mol. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 6. Formation of product ions **B**, **C**, **D**, and **E** from protonated compound **8**. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 7. Formation of diagnostic ions **K**, **L**, and **P** for compounds **4–5** and **G** for compound **8**. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 8. Formation of diagnostic ions **F**, **I**, **J**, and **Q**, which diagnose the absence of double bond between C-7'–C-8' and C-7–C-8, for compound **7**. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 9. Formation of diagnostic ions **N** and **O** for compound **2** and of diagnostic ions **H** and **M** for compound **3**. These ions indicate the presence of methoxyl and hydroxyl groups at C-3 and C-3'. **Enthalpies** and *Gibbs energies* are in kcal/mol.

SCHEME 10. Product ion $[7+H-\bullet CH_3]^+$ formation (m/z 400). **Enthalpies** and *Gibbs energies* are in kcal/mol. *Not observed in the product ion spectrum of protonated compound **2** (m/z 415).